

**A**

```

89.6   ATAATGGAAAGAAATCTGTTGACTCAGATTGGCTGCACCTTAAATTTCCCATAGTCCTATTGAAACTGTACCAGTAAAAATAAAGCCAGGAATGGATGGTCCAAAAG
NL4-3  -----C-----G-----C-----

89.6   TGAAACAATGGCCATTGACAGAAGAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAATTTCAAAAATGGGCCTGAGATCCATACAA
NL4-3  -T-----A-----

89.6   TACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTACAGAACTTAATAAGAGAACCAAGACTTCTGGGAAGTTCAATTAGGC
NL4-3  -----T-----T-----A-----

89.6   ATACCACATCCCGCAGGGTTAAAAAAGAAAAAATCAGTAACAGTACTGGATGGGTGATGCATATTTTTCAGTCCCTTAGATGAAGACTTCAGGAAGTACACTGCAT
NL4-3  -----T-----C-----C-----A-----T-----

89.6   TTACCATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAATACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAA
NL4-3  -----G-----GT-----

89.6   AATCTTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGACTTGTATGTAGGATCTGACTTAGAAAATAGGCAGCATAGACAAAATA
NL4-3  -----C-----T-----A-----

89.6   GAGGATCTGAGACAACATCTGTTGAAAGTGGGGTTTACCACACCAGACAAAAACATCAGAAAAGAACCTCCATTTCTTTGGATGGGTATGAAGCTCCATCCTGATAAAT
NL4-3  ---A-----G-----A-----C-----

89.6   GGACAGTACAGCCTATAAGTCTGCCAGAAAAAGACAGCTGGACTGTCAATGATATACAGAAAGTTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTACGAGGGATT
NL4-3  -----G-----C-----A-----A-----

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**B**

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1B7   ATAATGGAAAGAAATCTGTTGACTCAGATTGGCTGCACCTTAAATTTCCCATAGTCCTATTGAAACTGTACCAGTAAAAATAAAGCCAGGAATGGATGGCCAAAAG
1D1  -----

1B7   TTAAACAATGGCCATTGACAGAAGAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAATTTCAAAAATGGGCCTGAAAATCCATATAA
1D1  -----

1B7   TACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTACAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGGA
1D1  -----C-----

1B7   ATACCACATCCTTCAGGATTAATAAAGAAAAAATCAGTAACAGTACTGGATGGGTGATGCAATTTTTCAGTACCCTTAGATGAAGACTTCAGGAAGTACACTGCAT
1D1  -----T-----T-----

1B7   TTACCATACCTAGTATAAACAATGAAACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAAAGTAGCATGACAAA
1D1  -----

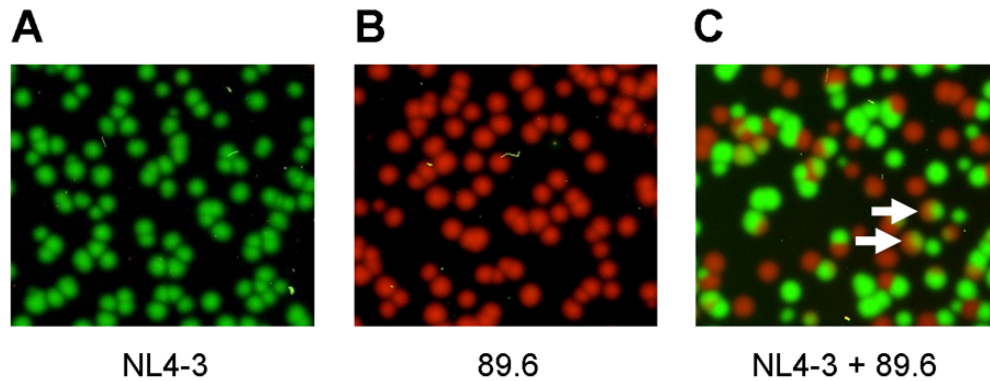
1B7   AATATTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAAATAGGCAGCATAGAACAAAATA
1D1  -----A-----

1B7   GAGGACTGAGACAACATCTGTTGAGGTGGGGTTTACCACACCAGACAAAAACATCAAAAAGACATCCATTCTTTGGATGGGTATGAAGCTCCATCCTGATAAAT
1D1  -----A-----C-----

1B7   GGACAGTACAGCCTATAAAGCTGCCAGAAAAAGAAAGTTGGACTGTCAATGACATACAGAAAGTTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTACGAGGGATT
1D1  -----

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**Figure S1. Sequence comparison between templates.** Two different HIV-1 strains 89.6 and NL4-3 (A) and two genetic variants 1B7 and 1D1 of HIV-1 strain WEAU (B) were compared to each other. The identical nucleotides are indicated by dash. The nucleotides that were used in the PASS assay to distinguish both templates from each other are indicated by boxes. The positions of each nucleotide used for PASS assay in the PCR amplicon are indicated above the base. The PCR primer sequences are indicated by underline.



**Figure S2. Detection of homoduplexes and heteroduplexes by PASS.** The NL4-3 (A) and 89.6 (B) were either amplified individually or as a mixture (C) by PCR. The PCR products were then subjected to PASS analysis. Each polony was annealed to primer 729 and SBE was performed with the Cy3-labeled NL4-3-specific nucleotide (green) and the Cy5-labeled 89.6-specific nucleotide (red). No heteroduplexes were detected in the PCR products amplified from 89.6 or NL4-3 template alone, while a high proportion of the polonies were heteroduplexes (indicated by arrow) in the PCR products amplified from the mixture of 89.6 and NL4-3.

**Table S1.** Recombination patterns and frequencies of the PCR amplicons with different thermocycles

Pattern							No. of each recombination pattern											
33	207	384	585	646	729		5 Cycles (697)		10 Cycles (433)		15 Cycles (606)		20 Cycles (420)		25 Cycles (442)		30 Cycles (592)	
							No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
+		-	-	-	-	-	--	--	--	--	--	--	--	--	22	4.98	29	4.90
-		+	+	+	+	+	--	--	--	--	--	3	0.71	27	6.11	35	5.91	
+	+	+		-	-	-	--	--	--	--	--	2	0.48	18	4.07	24	4.05	
-	-	+	+	+	+	+	--	--	--	--	--	--	--	9	2.04	28	4.73	
+	+	+	+		-	-	--	--	--	--	--	5	1.19	14	3.17	26	4.39	
-	-	-	-	+	+	+	--	--	--	--	--	3	0.71	11	2.49	17	2.87	
+	+	+	+	+	+		--	--	--	--	--	--	--	1	0.23	8	1.35	
-	-	-	-	-	+	+	1	0.14	--	--	--	--	--	5	1.13	9	1.52	
+	+	+	+	+	+		--	--	--	--	--	--	--	7	1.58	5	0.84	
-	-	-	-	-	-	+	--	--	--	--	1	0.17	1	0.24	4	0.90	6	1.01
-		+		-	-	-	--	--	--	--	1	0.17	--	--	1	0.23	8	1.35
+	-	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	5	0.84
-	-	-	+		-	-	2	0.29	--	--	1	0.17	--	--	--	--	7	1.18
+	+	+	-	+	+	+	--	--	--	--	1	0.17	--	--	--	--	5	0.84
-	-	-	+	+	+	+	1	0.14	--	--	--	--	--	--	--	--	1	0.17
+	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
-	-	-	-	-	+		--	--	2	0.46	--	--	1	0.24	--	--	--	--
-		+	+	-	-	-	--	--	--	--	--	--	--	1	0.23	5	0.84	
+	-	-	-	+	+	+	--	--	--	--	--	--	--	2	0.45	3	0.51	
-	-	+	+	+	+	+	1	0.14	--	--	--	--	--	2	0.45	1	0.17	
-	-	-	-	-	+		--	--	--	--	--	--	--	--	--	--	2	0.34
-	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	2	0.34
+	-	-	-	-	+	+	--	--	--	--	--	--	--	--	--	--	2	0.34
-	-	+	+	+	+	+	--	--	--	--	--	--	--	1	0.23	1	0.17	
+	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	2	0.34
-	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	3	0.51
+	-	-	-	-	-	+	--	--	--	--	--	--	--	1	0.23	--	--	
+	-	+	+	-	-	-	--	--	--	--	--	--	--	--	--	--	2	0.34
-	+	+	-	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	-	-	-	+	+	+	1	0.14	--	--	1	0.17	--	--	--	--	1	0.17
+	-	-	-	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	-	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	-	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	2	0.34
+	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	+	+	+	+	+	+	--	--	--	--	--	--	--	--	--	--	1	0.17
+	+	+	+	+	+	+	1	0.14	--	--	--	--	--	--	--	--	--	--
-	+	+	+	+	+	+	1	0.14	--	--	--	--	--	--	--	--	--	--
Total							8	1.15	2	0.46	5	0.83	15	3.57	126	28.51	247	41.72

The plus sign represents the bases specific for 89.6 and the mini sign represents the bases specific for NL4-3. The red vertical bar indicates the purgative recombination breakpoint. The number of PCR amplicons used for analysis are indicated within brackets.

**Table S2.** Recombination patterns and frequencies of the PCR amplicons with different input numbers of templates

Pattern							No. of each recombination pattern							
33	207	384	585	646	729	$2 \times 10^1$ copies (487)		$2 \times 10^3$ copies (519)		$2 \times 10^5$ copies (518)		$2 \times 10^7$ copies (592)		
						No.	%	No.	%	No.	%	No.	%	
+	-	-	-	-	-	--	--	1	0.19	12	2.32	29	4.90	
-	+	+	+	+	+	--	--	--	--	17	3.28	35	5.91	
+	+	-	-	-	-	--	--	--	--	5	0.97	24	4.05	
-	-	+	+	+	+	--	--	--	--	4	0.77	28	4.73	
+	+	+	-	-	-	--	--	--	--	1	0.19	26	4.39	
-	-	-	+	+	+	--	--	--	--	14	2.70	17	2.87	
+	+	+	+	-	-	1	0.21	1	0.19	2	0.39	8	1.35	
-	-	-	-	+	+	--	--	--	--	1	0.19	9	1.52	
+	+	+	+	+	-	--	--	--	--	2	0.39	5	0.84	
-	-	-	-	-	+	--	--	--	--	4	0.77	6	1.01	
-	+	-	-	-	-	--	--	--	--	--	--	8	1.35	
+	-	+	+	+	+	1	0.21	--	--	--	--	5	0.84	
-	-	+	-	-	-	--	--	--	--	1	0.19	7	1.18	
+	+	-	+	+	+	--	--	1	0.19	1	0.19	5	0.84	
-	-	-	+	-	-	--	--	1	0.19	1	0.19	1	0.17	
+	+	+	-	+	+	--	--	--	--	--	--	1	0.17	
-	-	-	-	+	-	--	--	--	--	2	0.39	--	--	
-	+	+	-	-	-	--	--	--	--	--	--	5	0.84	
+	-	-	+	+	+	--	--	--	--	--	--	3	0.51	
-	-	+	+	-	-	3	0.62	--	--	--	--	1	0.17	
-	-	-	+	+	-	--	--	--	--	--	--	2	0.34	
-	+	+	+	-	-	--	--	--	--	--	--	2	0.34	
+	-	-	-	+	+	--	--	--	--	--	--	2	0.34	
-	+	+	+	+	-	--	--	--	--	--	--	1	0.17	
+	+	-	-	-	+	--	--	--	--	--	--	2	0.34	
-	+	+	+	+	-	--	--	--	--	1	0.19	3	0.51	
+	-	+	-	-	-	--	--	--	--	--	--	2	0.34	
-	+	-	+	+	+	--	--	--	--	--	--	1	0.17	
+	-	-	+	-	-	--	--	--	--	--	--	1	0.17	
+	-	-	+	+	-	--	--	--	--	--	--	1	0.17	
+	-	+	+	-	-	--	--	--	--	--	--	1	0.17	
+	-	-	+	+	-	--	--	--	--	--	--	1	0.17	
+	+	-	+	+	-	--	--	--	--	--	--	2	0.34	
+	+	+	-	+	-	--	--	--	--	--	--	1	0.17	
-	-	-	+	-	+	--	--	--	--	--	--	1	0.17	
-	+	-	-	-	+	--	--	--	--	--	--	1	0.17	
Total						5	1.03	4	0.77	68	13.13	247	41.72	

The plus sign represents the bases specific for 89.6 and the mini sign represents the bases specific for NL4-3. The red vertical bar indicates the purgative recombination breakpoint. The number of PCR amplicons used for analysis are indicated within brackets.

**Table S3.** Recombination patterns and frequencies of the PCR amplicons with different extension time

Pattern						No. of each recombination pattenin in different minutes (min)							
33	207	384	585	646	729	1 min (348)		2 min (401)		4 min (405)		8 min (438)	
						No.	%	No.	%	No.	%	No.	%
+	-	-	-	-	-	28	8.05	8	2.00	13	3.21	5	1.14
-	+	+	+	+	+	15	4.31	10	2.49	11	2.72	2	0.46
+	+	-	-	-	-	18	5.17	13	3.24	9	2.22	--	--
-	-	+	+	+	+	16	4.60	11	2.74	4	0.99	1	0.23
+	+	+	-	-	-	16	4.60	3	0.75	3	0.74	3	0.68
-	-	-	+	+	+	10	2.87	11	2.74	2	0.49	1	0.23
+	+	+	+	-	-	4	1.15	3	0.75	1	0.25	--	--
-	-	-	-	+	+	1	0.29	5	1.25	1	0.25	--	--
+	+	+	+	+	-	7	2.01	2	0.50	3	0.74	2	0.46
-	-	-	-	-	+	4	1.15	5	1.25	--	--	5	1.14
-	+	-	-	-	-	2	0.57	2	0.50	--	--	--	--
+	-	+	+	+	+	--	--	2	0.50	--	--	--	--
+	+	-	+	+	+	1	0.29	--	--	1	0.25	--	--
+	+	+	-	+	+	2	0.57	--	--	--	--	--	--
+	+	+	+	-	+	1	0.29	--	--	--	--	--	--
-	-	-	-	+	-	--	--	1	0.25	--	--	--	--
-	+	+	-	-	-	--	--	4	1.00	--	--	--	--
+	-	-	+	+	+	1	0.29	1	0.25	--	--	--	--
+	-	-	-	+	+	--	--	--	--	--	--	1	0.23
-	+	+	+	-	-	1	0.29	--	--	1	0.25	--	--
+	-	-	-	-	+	--	--	--	--	1	0.25	--	--
-	+	+	+	+	-	1	0.29	--	--	--	--	--	--
+	-	+	+	+	-	3	0.86	--	--	--	--	--	--
-	-	-	+	-	+	--	--	--	--	1	0.25	--	--
Total						131	37.64	81	20.20	51	12.59	20	4.57

The plus sign represents the bases specific for 89.6 and the mini sign represents the bases specific for NL4-3. The red vertical bar indicates the purgative recombination breakpoint. The number of PCR amplicons used for analysis are indicated within brackets.